SYMPOSIUM

INDIVIDUALIZED INFECTION MEDICINE – THE FUTURE IS NOW

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SELECTED ABSTRACTS
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MALARIA AND WEST-NILE VIRUS CO-INFECTION AMONGST FEBRILE PATIENTS ATTENDING A TERTIARY HOSPITAL IN ABUJA, NIGERIA

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Background: Malaria and West Nile fever has ubiquitous distribution in Africa. Many febrile patients are most times underdiagnosed or misdiagnosed with malaria due to striking similarities, such as fever shared by malaria and certain arboviral infections. Clinical symptoms of WNV fever often overlap with other agents of febrile illnesses. Over the years, the geographical range of WNV activity has increased and the virus has become established even in non-endemic areas where it has not been previously detected.
Methods: This serological-survey investigated the prevalence of anti-WNV IgM and Malaria among patients with febrile illnesses at Gwagwalada metropolis, Abuja. Between the period of May and August 2016, a total of 171 participants attending the University of Abuja Teaching Hospital were recruited for the study. Serum samples were immediately harvested, stored and analyzed using the indirect ELISA for anti-WNV IgM antibodies using kits endorsed by the World Health Organization and also Microscopy and RDTs for Malaria. Socio-demographic variables and clinical data was gotten using a self-administered interviewer-based questionnaires.
Results: Out of the 171 febrile participants, the overall prevalence of WNV IgM antibodies was 66.1%. Significant association was observed in prevalence of WNV IgM and Malaria/WNV co- infection ($p< 0.5$). Sixty two (54.9%) of WNV seropositive females and 51/113 (45.1%) seropositive males was recorded. With regards to participants' knowledge, attitude and practice towards preventive measures against WNV, significant association was observed between the WNV IgM seropositivity and the use of mosquito repellants ($p =0.016$).
Conclusions: Findings from this study necessitate the need for routine diagnosis and surveillance of WNV as possible agents of febrile illness in Nigeria. More so, infected patients should be closely monitored in order to detect possible associated sequelae.

2 UNTARGETED LC-MS METABOLOMICS DIFFERENTIATES BETWEEN VIRULENT AND AVIRULENT CLINICAL STRAINS OF *PSEUDOMONAS AERUGINOSA*

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The opportunistic Gram-negative pathogen *Pseudomonas aeruginosa* is a major threat for patients suffering from cystic fibrosis (CF), pneumonia, or wound infections. Different *P. aeruginosa* strains can cause acute infections or persist chronically in predisposed individuals. In the clinical setting, knowledge about the virulence of a given strain is needed as early as possible after the diagnosis as it can inform the therapeutic decision as well as the prognosis of the infected patient. Because the *P. aeruginosa* genome is highly conserved, it is more promising to differentiate strains based on functional genomics data. Compared to many other pathogenic bacteria, *P. aeruginosa* relies quite strongly on secondary metabolites for the exertion and regulation of its virulence. These small molecules, e.g. phenazines, rhamnolipids, homoserine lactones and alkyl quinolones, can be detected and quantified by liquid chromatography coupled to mass spectrometry and differential abundance of these metabolites can be identified.
We applied an untargeted LC-MS metabolomics approach on cell extracts of *P. aeruginosa* clinical strains and used multivariate statistics and machine learning approaches to identify sets of metabolites that differentiate between virulent and avirulent/persistent strains whose pathogenic potential was determined by the use of the *Galleria mellonella* virulence assay. Among the differentially abundant metabolites are, e.g., alkyl quinolone quorum sensing signal molecules but also metabolites less directly linked to virulence along with previously undescribed compounds. We were able to generate and initially validate predictors based on the metabolomics data that are under investigation as putative biomarkers for the differentiation between virulent and avirulent strains of *P. aeruginosa*.

CONTRACEPTION USE AND PREFERENCE AMONG HIV DISCORDANT COUPLES IN YAOUNDÉ CENTRAL HOSPITAL, CAMEROON: A CROSS SECTIONAL SURVEY

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Introduction: Evidence in sub-Saharan Africa indicates that most of HIV discordant couples want more children despite their HIV status. Since the usage of contraception remains as women concern in most of African countries, the aim of this study was to investigate contraception preferences among women infected with HIV in discordant couple to provide effective and individualized reproductive healthcare in Cameroon.
Methods: We performed a cross-sectional study using structured questionnaire to explore participant's patterns that included the family planning services, preferences and its use, and knowledge related to HIV infection of negative sexual partner. Bivariate and multivariate analyses were conducted to fit associated and predictive patterns of contraception preference.
Results: Overall, 94 HIV-positive pregnant women aged 30.70±5.50 years living with HIV-negative partners were recruited from the different areas of the central region of Cameroon. Three- fourths were aware of the effectiveness of modern contraceptives including condoms, however only 28% had experienced modern contraption. 98% preferred to use traditional methods associated with infrequent condoms use. Multiple sociodemographic factors (marital status, group age, educational level, religion, occupation) affected contraceptive method preferences and its use ($P<0.05$). These factors are the landmarks to predict discordant couples' behavior in HIV infection disclosure, discussion and decision making for contraception, preventing mother to-child transmission and HIV-negative partner infection ($P<0.05$).
Conclusions: Despite the awareness of participants related both on contraception methods and HIV infection, participants faced societal, cultural and demographic barriers to make own decision for contraception use. Promoting individualized family planning services and given the entire range of contraception options may help women living with HIV to choose for effective ones and consequently reduce newly HIV infections.

4 THE ROLE OF PHENOLIC COMPOUNDS ON CANDIDA INFECTIONS: AN EYE-OPENING LOOK

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Opportunistic infections have deserved a special attention among medical and scientific communities, namely those involving *Candida* species. Despite their presence as commensal microorganisms, alarming rates of local and systemic infections have been observed, varying from moderate to severe impact. Further, currently available antifungal drugs have progressively lost effectiveness, pointing urgently the problem of microorganisms with acquired-resistance. Natural matrices are secularly used for numerous purposes, and the particular contribution of phenolic extracts/compounds have been highlighted and directly correlated with other bioactive effects. Thus, the main focus of this work was to access the anti-*Candida* activity of phenolic extracts/compounds and to compare with their antioxidant potential, also providing the phenolic composition analysis in bioactive extracts. A direct correlation between phenolic composition and biological potential was also established, and the mode of action of most promissory matrices investigated.

Among studied matrices (i.e. anise, oregano, coriander, thyme, sage, licorice), licorice was the most promising, exerting both excellent anti-*Candida* and antioxidant effects. More interestingly, licorice exerted candidacidal effects not only against planktonic cells, but also against its biofilms counterparts. Concerning to antioxidant potential, it mainly acted as strong lipid peroxidation inhibitor and free radical's scavenger. Both biological effects were positively correlated with phenolic composition, namely with the content in flavones (apigenin derivatives), flavanones (liquiritin derivatives) and a methylated isoflavone (formononetin-7-O-apiosylglucoside). Then, the biological activity of the most abundant phenolic compounds was also assessed, including mixtures of them, but no significant effects were stated. It means that probably the most abundant compounds were not the main contributors to the observed effects, but instead through their interaction with minor compounds, acting in synergism. The effect of licorice extract on *Candida* cells was confirmed afterwards, by flow cytometry and transmission electron microscopy. Licorice exerted fast and irreversible effects in *Candida* cells, affecting primarily cell membrane permeability. However, no cytotoxic effects were stated at effective doses.

Based on the current findings, licorice extract possesses all the required premises to assess *in vivo* efficacy, to establish therapeutic and/or prophylactic doses aiming to improve *Candida* infections control.

INDIVIDUALIZED INFLUENZA TREATMENT VIA ADAPTIVE OSELTAMIVIR DOSES

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Influenza A virus (IAV) is a latent global threat to the human health. In view of the risk of pandemics, prophylactic and curative treatments are essential. Oseltamivir is a neuraminidase inhibitor efficiently supporting recovery from influenza infections. Current common clinical practice is a constant drug dose administered at regular time intervals irrespective of the bidirectional interplay between patient's immune system and the virus. We aim to propose an adaptive and individualized oseltamivir scheduling. We combined the data-validated mathematical model for IAV infections with a pharmacokinetics (PK)/pharmacodynamics (PD) model of oseltamivir. Next, depending on the individual dynamics of the immune system (CTL counts) and the virus (by PCR-based methods), the adaptive drug dose is calculated systematically via feedback control methods. Quantification of the virological efficacy reveals that treatment with adaptive control-based drug scheduling is able to increase drug virological efficacy or reduce the drug dose while keeping the same virological efficacy.

We believe this study paves the ground for optimized drug dose in the field of personalized medicine. Actually, treating influenza with adaptive adjustment of the drug dose individually (i) reduces the possible side-effects of the drug and (ii) economically results in the reduction of stored oseltamivir for the prevention of pandemics.

6 COMBINED EFFECT OF PHOTODYNAMIC AND SONODYNAMIC THERAPY ON EHRLICH ASCITES TUMOR IMPLANTED IN MICE

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There are a host of risk factors aside from infectious disease that affect people in developing countries, such as malnutrition, poor sanitation and cancer. More recently, infections with certain viruses, bacteria, and parasites have been recognized as risk factors for several types of cancer in humans. Treatment of Cancer is one of the major problems which facing the scientists many years Ago. Chemotherapy is the most common cancer treatment, patient who get this kind of treatment lead to destroys the cells of the body both of normal and malignant cells, which cause side effects such as Renal dysfunctions, blood toxicity, Lymphocytopenia or Leucopenia other symptoms like vomiting, fatigue, diarrhea, loss of hair and nails. Worldwide, infections are linked to about 20% of cancers.

Sono-photodynamic therapy (SPDT) treat malignant tumors with minimal side effects, which involves three individually non-toxic components that are combined to induce cellular and tissue effects. produce reactive oxygen species (ROS), cytotoxic agents that can inactivate tumor cells. a combined treatment of near infrared laser and Ultrasound waves with and without sensitizer was investigated. Porphyrin derivative was used as a sono-photosensitizer Hemato-porphyrin dihydrochloride (HPD) and two sources of energy were used; namely infrared laser with three frequency levels and Ultrasound (pulsed and continuous wave mode).

Tumor bearing animals were divided into seven groups, each of 10 mouse, Two control groups, and 5 sub-groups. The site of the tumor in two sub-groups was irradiated with IR laser with different frequencies in presence and absence of HPD. Another 2 sub-groups were irradiated with pulsed ultrasound or continuous ultrasound wave mode in presence and absence of HPD. One group injected IP with HPD then exposed to infrared laser of 7000 Hz. followed by exposure to continuous wave ultrasound.

Treatment with sensitizer alone has no effect. The cytotoxic effect of IR laser increases with increasing of laser energy. Decreasing in the normalized tumor volume, the best effect at 7000 Hz infrared laser in the presence of the HPD. The cytotoxic effect of continuous wave ultrasound was more cytotoxic than that in pulsed wave. Combined treatment of IR laser at 7000 Hz and continuous wave ultrasound in the presences of sensitizer was more cytotoxic and effective. Evaluation of the ultrastructural changes by electron microscopy reflected inertial morphological destruction to nuclear and cell organelles.

RISK FACTORS ASSOCIATED WITH HIV AND STIS INFECTION AMONG FOREIGNER MALE SEX WORKERS IN NORTHERN THAILAND

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Introduction: Male sex worker (MSW) is one of the most favorite job for ethnic minority youths from outside Thailand. There are working in high tourist attractive area such as Chiang Mai. Due to low skill in Thai communication and unpermitted work in Thailand lead them to be a new vulnerable for STIs infections including HBV, HIV, etc. The study aimed to investigate the situation and to determine factors associated with HBV and HIV co-infection among ethnic MSW in northern Thailand.
Materials and Methods: A cross-sectional study design was conducted. MSWs were the study population. A simple random sampling techniques was applied. A validated questionnaire and 5 ml blood specimen were used research instruments. Interview and specimen collection as done in a private and confidential room after obtaining informed consent. Logistic regression was used to detect the association at the alpha=0.05.
Results: 250 MSWs were recruited into the study, average age at 23.2 years old, 55.6% were Chan, 96.4% were Buddhist, 96.4% were uneducated, 18.4 were married, 70.8% had income <5,000 baht/month (166$US), and 67.2 had no Thai ID card. 96.8% had sex at age <20 years, 23.2% had first sex with male, 62.8% were staring work as MSW at age < 20 years old, 94.0% had number of sex partner <20 person/week, 81.6% had partner MSM only, 27.0% did not use condom. Prevalence of HIV was 3.6%, and 21.0% were HBsAg, 1.78 were confections. In multiple logistic regression found that number of partner (OR=1.78, 95%CI=1.23-2.03), age at starting sex worker (OR=2.44, 95%CI=1.54-4.77), and did not use condom (OR=2.56, 95%CI=2.01-4.32) were associated with HIV and HBV confection.
Conclusion: A new preventive STIs infection program should be promoted for the ethnic MSW in northern Thailand including condom use and regularly health check.

SELECTED ABSTRACTS

presented as poster

IPSC-DERIVED PHAGOCYTES AS A CELLULAR THERAPEUTIC TO TREAT BACTERIAL INFECTIONS

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According to the World Health Organization (WHO), infectious diseases of the lower respiratory tract remain among the top five causes of death with 3.2 million reports in 2015. Especially the increasing number of severe infections with multi-drug-resistant pathogens highlights the need for alternative treatment options. Given the pivotal role of phagocytes and especially alveolar macrophages in pulmonary immunity, we aim to develop a novel, cell-based treatment strategy to enhance endogenous pulmonary immunity and fight bacterial airway infections.

To produce sufficient quantities of therapeutic phagocytes, we made use of induced pluripotent stem cell (iPSC) technology and established the large-scale production of human iPSC-derived macrophages (iPSC-MAC) in industry-compatible bioreactors. Generated iPSC-MAC shared important phenotypic and transcriptional hallmarks with professional phagocytes derived from peripheral blood. Moreover, iPSC-MAC efficiently phagocytosed *Pseudomonas aeruginosa* or *Staphylococcus aureus in vitro*, secreted important pro-inflammatory cytokines, and up-regulated defined gene sets associated with activated innate immunity and pathogen clearance.

Even more importantly, pulmonary transplantation of iPSC-MAC rescued immunodeficient mice from established pulmonary *P. aeruginosa* infections as demonstrated by significantly reduced disease scores, stable body temperatures, and normal lung function in treated animals. IPSC-MACs exhibited therapeutic activity as early as 4-8 hours after administration and dramatically reduced the bacterial load within 24 hours of infection.

In summary, we here introduce an innovative cell-based and antibiotic independent treatment strategy targeting bacterial respiratory infections. The pronounced therapeutic effect observed in this proof-of-concept study points to a broad applicability of iPSC-MACs for the treatment of bacterial infections.

9 IDENTIFICATION OF HETEROZYGOUS NFKB1 VARIANTS IN CVID COHORT USING TARGETED NEXT GENERATION SEQUENCING

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Background: CVID is characterized by recurrent bacterial infections of respiratory tract. It is the most prevalent symptomatic antibody deficiency syndrome. NFκB1 encodes the transcription-factor precursor p105 protein which can undergo cotranslational processing by the 26S proteasome to produce the active subunit p50 (canonical NF-κB pathway).
Methods: Using targeted next generation sequencing, we identified five heterozygous novel NFκB1 mutations in a cohort of 180 patients with CVID.
Results: Two novel heterozygous NFκB1 truncated mutations detected in two affected brothers from non- consanguineous parents and one unrelated CVID patient (p.Ser338Leufs*94; p.S302Ffs*7). These mutations are predicted to lead to non-functional proteins, which might undergo rapid decay, thus resulting to p50 haploinsufficiency. Another CVID patient harbored a NFκB1 frameshift mutation (p.Ile567Asnfs*6), which is predicted to lead to aberrant p50 or loss of p105. In two further patients we identified NFκB1 heterozygous splice-donor-site mutation (c.1210+1G>A) causing in-frame skipping of exon 12 and a point mutation with unknown effect.
Conclusions: Heterozygous NFκB1 mutations seem to be prevalent in CVID patients and may cause monogenic CVID with very different clinical manifestations and onset.

PARADOXICAL ASPECT OF SIROLIMUS: ANTIVIRAL CD8+ T CELLS SHOW POTENT INCREASED FUNCTIONALITY UNDER mTOR INHIBITION

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Introduction: Cytomegalovirus (CMV) infection and reactivation remains one of the most important complications in transplantation medicine, particularly in patients undergoing intense immunosuppression (IS). Recent observations indicated that patients treated with mTOR inhibitor, e.g. sirolimus, may exhibit favorable outcomes of CMV infection following transplantation. This study was designed to elucidate this effect through investigation of the role of mTORC1 signaling in CMV-specific cytotoxic CD8+ T cells (CTLs).
Methods: CD8+ T cells were stimulated with artificial antigen-presenting cells (aAPCs) loaded with CMVpp65 peptide. The effect of sirolimus on the proliferative capacity, phenotype and functionality of CTLs was determined. Furthermore, we applied next-generation sequencing (NGS) to monitor dynamics of TCR repertoires under the influence of sirolimus as well as detection of signaling pathways and expression of target and effector molecules was assessed.
Results: Despite the inhibited expansion, sirolimus induced strong T-cell activation, had no effect on the effector memory phenotype and significantly increased antigen-specific effector T-cell response. Key elements of T-cell activation and function such as (1) dynamics of TCR repertoires, (2) phosphorylation of kinases and proteins, and (3) expression of miRNAs and genes were differently affected under sirolimus treatment, indicating their influence in the improved functionality.
Conclusion: In contrast to expectations, we showed improved functional qualities of CMV-CTLs exposed to sirolimus. Modulating the environmental cues during CTL formation by IL-2R driven STAT-5 signaling under mTORC1 inhibition allows the fine- tuning of CTL programming to promote antiviral T-cell response with stable dynamics of TCR repertoires. This study provides help for further individualization of IS therapy, indicating a potential benefit of sirolimus in patients with elevated risk of CMV infection.

11 INDIVIDUAL NK CELL IMMUNITY IN HCV PATIENTS UNDER DAA THERAPY

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NK cell effector functions play a pivotal role in the direct defense against tumors and viral infections. During chronic hepatitis C (HCV) infection, however, NK cell responses are impaired paving the way for life-threatening liver cirrhosis or hepatic carcinomas. Recently, the approval of direct acting antivirals (DAAs) has revolutionized HCV therapy. DAA application results in complete viral clearance, but the molecular understanding of NK cell immunity along DAA therapy remains undefined.

We have established a clinical proteome workflow to characterize primary human NK cell immunity in HCV patients: NK cells are sorted from peripheral blood using flow cytometry and accurate mass spectrometry is then used to monitor NK cell functions systematically. In a pilot study the immune status of individual HCV patients was characterized before, along, as well as one year after Harvoni™ therapy (i.e. combined DAAs sofosbuvir and ledipasvir). Preliminary data revealed NK cell functions that were rescued after Harvoni™ therapy in each of the investigated HCV patients. Most notably, our data indicate patient-specific and incomplete restoration of numerous NK cell features after HCV clearance. This includes RNA-binding proteins possibly being instrumental for HCV to establish chronic infections.

Ultimately our project aims to complement knowledge on the molecular basis of viral immune evasion, but already now complements knowledge on the NK immune status of individual patients. Thus, a more systematic profiling of HCV patients might improve risk management after DAA/Harvoni™ treatment and, in the long run, support the identification of prognostic biomarkers and novel drug targets suitable for immune-modulatory therapies.

THERAPEUTIC POTENTIAL OF INTRABODIES IN CANCER THERAPY

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Intracellular antibodies are now capable to knockdown virtually every intracellular protein and desired epitope. ER intrabodies expressed as scFv's in the ER are capable to inhibit proteins passing the ER, such as cell surface receptors, intracellular receptors, Golgi located or secretory proteins. In addition to this inhibitory machinery, cytosolic and nuclear proteins can be inhibited by single domain antibodies comprising only the variable domain of the heavy chain. They are mainly derived from camels or sharks. ER and cytosolic intrabodies are able to inhibit tumor growth in cell culture and mice. This indicates a therapeutic potential of intrabodies in cancer therapy.

The main challenge today is to target the intrabody gene or intrabody protein/mRNA specifically and efficiently to the tumor cells. New improvements of cell specific adeno-associated viruses and cell-specific nanoparticles are promising and might pave the way to translate intrabodies into clinics in the next decades. Demonstrated will be an overview of recent developments with intrabodies in xenograft tumor mouse models. In addition an example of recently published ER intrabodies inhibiting Golgi located polysialyltransferases in rhabdomyosarcoma tumor cells will be shown. Inhibition of the cell surface expression of polysialylated NCAM leads to delayed metastasis if tumor cells stable expressing the intrabodies were xenografted into C57BL/6 J RAG-2 mice.

13 INDIVIDUALIZED HIV-MEDICINE – EARLY DETECTION OF RESPIRATORY DISORDERS IN HIV PATIENTS

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Background: Aging with HIV is the success of suppressive antiretroviral therapy but leads to high burden of co-morbidities and non-AIDS defining events. This may be due viral persistence and chronic inflammation and requires individualized and patient-oriented approaches for long-term care of HIV infected patients. The risk for obstructive pulmonary disease is increased in HIV patients. Our aim was to assess the prevalence of COPD vs. asymptomatic obstruction in an unselected cohort of HIV-positive patients.
Methods: 623 consecutively recruited patients from an HIV outpatient clinic were prospectively assessed by spirometry, standardized questionnaire and alpha-1 antitrypsin testing. Restriction and obstruction were classified by Lower Limit of Normal. Descriptive statistics and Chi-square test were used (sig. $p < 0.05$).
Results: 75% of patients were male, 84% Caucasian, main transmission risks were MSM (35%) and heterosexual contacts (21.3%). Mean age was 50 ± 13 years. 44% of patients reported current, 22.6% former smoking. Overall, 31% of patients had an abnormal spirometry, mostly evidence for obstructive lung disease. Only 58% of all patients with persistent obstruction reported chronic symptoms consistent with COPD (COPD). 39% of COPD reported chronic cough and sputum production, while 80.4% reported dyspnea while walking fast. 19.4% of all patients without obstruction (no-OBS) reported a cold within the past 3 weeks, 29.4% of asymptomatic patients with obstruction (OBS) and 58.7% of COPD. Of all COPD patients, 10.9% were hospitalized during the past year due to an exacerbation. Prevalence of ventilatory disorders increased with age. While only 11% of <40-year-olds had obstruction, 26% of the >50-year-olds did. Patients with obstruction were significantly more often ex- or current smokers (92% vs. 61%, $p < 0.001$) or exposed to passive smoking (40% vs. 26%, $p = 0.006$). While 84.4% of COPD were advised to quit smoking by their doctor, only 53% of no-OBS were advised to do so. Only 52% of COPDs had a documented COPD diagnosis prior to the study.
Conclusion: One third of all screened patients had abnormal lung function testing and almost half of patients with persistent obstruction did not report symptoms and are therefore not yet classified as COPD. Progression rates to COPD in these patients are unknown. Our study supports the need of individualized strategies to identify HIV patients most at risk for comorbidities and premature aging processes. We propose a comprehensive approach for individualized HIV-infection medicine based on pathogen-related information, patient-related genetic, biomarker and cohort data, and modern physician-patient communication tools.

RESTORING INNATE IMMUNITY - CHARACTERIZATION OF AIRWAY AND BONE MARROW DERIVED MACROPHAGES IN A MOUSE MODEL OF CYSTIC FIBROSIS

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Chronic lung infections are a major threat to the constitution of cystic fibrosis (CF) patients. An increasing number of publications indicate that mutations of the *cystic fibrosis transmembrane conductance regulator* (CFTR) gene not only cause malfunctions of this sodium and bicarbonate channel in epithelial cells but in myeloid cells, such as macrophages, as well (Bruscia & Bonfield; J Innate Immun; 2016). Defective alveolar macrophages might contribute to an impaired innate immune response and chronic lung infections in CF. In our project, bone marrow transplantation in a CF mouse model and subsequent characterization of transplanted wild type (WT) cells after engraftment in the lungs of chimeric mice were performed. Furthermore, alveolar macrophages of healthy wild type and CF mice were analyzed regarding their lysosomal acidification.

Transplantation of hematopoietic stem cells from WT mice in a CF mouse model revealed beneficial effects in the chimeric mice (CF^{B6}) under infectious conditions, e.g. lower lung bacterial numbers and increased survival compared to mice that had received isogenic stem cells (CF^{CF}). Tracked with the leucocyte surface marker antigen CD45, almost complete replacement of the original CF macrophages with successfully engrafted WT cells was observed.

Confocal live cell imaging with the fluorescent, pH-sensitive dye LysoSensor Green DND-189 (Zhang *et al*. 2010) revealed significantly less acidic pH in lysosomes of CF macrophages compared to healthy ones, confirming the hypothesis of malacidification in CF phagocytes.

In summary, our results strengthen the hypothesis of CFTR playing an important role also in non- epithelial cells such as phagocytes. Thinking this ahead, transplantation of patient-derived stem cells or macrophages might improve the innate immune answer in CF patients and help fighting against their chronic lung infections.

15 DIGITAL TOOLS FOR INDIVIDUALISED INFECTION MEDICINE

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Individualised infection medicine requires systems that can adjust fast to upcoming new topics and emerging pathogens. Also, highly flexible approaches of addressing the individual's conditions and abilities to participate in clinical and epidemiological research are needed. The rapidly developing framework of eHealth and mHealth (electronic and mobile health) offers various options to meet these needs.
Within the digital framework, we develop a mHealth tool, called personalised assessment of acute infections application (PIA), with the aim of facilitating real-time reporting of acute, transient infections including daily monitoring of disease progression and medication taken (scan of PZN or photo).
Programming principles focus on German data protection requirements and IT security as well as easy and intuitive usability (user-cantered design). The modular design offers the opportunity to address new research topics quickly without the need to programme a completely new tool but rather by adjusting the existing one. The use of conditional questionnaire facilitates inclusion of items directed only on subpopulations, e.g. individuals with certain conditions. For participants not using mobile digital devices we offer a web-based tool with identical functionalities.
The first application of PIA will take place within the German National Cohort. Here, we will focus on the reporting of symptoms of acute respiratory, gastrointestinal, urinary tract infections among men and women, and on bacterial vaginosis among women. In the case of respiratory infections PIA will ask study participants to take a nasal swab (self-sampling) to be send to multiplex PCR analysis of viruses. The results will be fed back real-time into PIA via HL7.
Future developments will include the integration of gamification elements, e.g. for the assessment of neurocognitive conditions. Gamification is also used to enhance adherence to the use of the mobile application.

HUMANIZED MICE RECONSTITUTED WITH MATURE T AND B CELLS FOR TESTING NOVEL IMMUNE MODULATORY STRATEGIES AGAINST EPSTEIN-BARR-VIRUS INFECTION AND LYMPHOMA DEVELOPMENT

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Introduction: Immune compromised hosts after stem cell transplantation or after solid organ transplantation are highly susceptible to Epstein-Barr-Virus (EBV) infections and reactivations. Besides the EBV impinged complications of a post-transplant lympho proliferative disease (PTLD), EBV is associated with lymphoma development. Until now, no vaccines and EBV-specific monoclonal antibodies are marketed. Due to the fact that EBV has a tropism only for humans and for some monkeys, *in vivo* testing of these immune modulators is difficult. Thus, we seek to establish humanized mouse models with adoptive immune responses to model EBV infection and test new therapies.

Methods: Nod.Rag.Gamma (NRG) were transplanted with cord blood $CD34^+$ hematopoietic stem cells and 15-17 weeks after HSCT used for challenge with EBV laboratory strains (B95.8/EGFP or B95.8/fLuc). Spatio-temporal distribution of virus infection was monitored with non-invasive intravital imaging (IVIS) over time. Mice were monitored for body weight, for the composition of immune cells and for tumor formation. The composition of immune cells was monitored for 10 weeks post infection in blood and at final time point furthermore in different immunological organs via flow cytometry.

Results: Infections in the spleen were detectable by IVIS one week post-infection, followed by a rapid increase of the bioluminescent signal in the consecutive weeks. At euthanasia, infection was detectable by IVIS in the spleen, liver, kidney, pancreas, lymph nodes and salivary glands. Monitoring the composition of immune cells in peripheral blood revealed that within 5 weeks post-infection mice

started to lose $CD19^+$ B cells, followed by a sharp increase in $CD8^+$ T cells a few weeks later. This effect was even more pronounced in mice which showed tumor formation. After sacrificing the mice 10 weeks post EBV infection we observed tumors in spleen, liver, kidney and pancreas. Analysis of splenocytes and other lymphatic tissues showed a dramatic expansion of $CD8^+$ T cells with high expression of PD-1, particularly in mice developing tumors, indicating an exhausted phenotype.

Conclusions: This robust model can be used in the future to test cell therapies, monoclonal antibodies and check-point inhibitors against EBV and EBV-related malignancies.

17 EFFICACY OF QS INHIBITORS AS AN ADJUNCTIVE TREATMENT AGAINST *P. AERUGINOSA* INFECTIONS IN BIOTIC BIOFIMS AND SUB-CHRONIC MURINE INFECTIONS

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Pseudomonas aeruginosa (PA) infection in patients with chronic lung diseases such as cystic fibrosis or bronchiectasis is associated with loss of lung function, morbidity, and mortality. Biofilm formation prevents bacterial clearance and favors persistence and increased tolerance towards antibiotics. Quorum sensing (QS) plays a critical role in virulence factor production, motility and biofilm formation and is a promising target for anti-virulence drugs. Aim of the study was to assess the efficacy of novel QS inhibitors (QSI) in a human epithelial cell culture as well as an *in vivo* mouse model of PA infection.

Air liquid interface-cultivated A549 cells were infected with PA14 in presence of QSI or vehicle. After 1h of infection, the inoculum was removed and cells were incubated with QSI or vehicle for up to 24h. Additionally, co-treatment with tobramycin (TOB) or vehicle was performed starting at 5h post infection (p.i.). Cell viability, bacterial load, and pro-inflammatory immune response were analyzed at 24h p.i. Further, a sub-chronic murine pulmonary infection was used. C57BL/6 mice were infected with an alginate-supplemented PA inoculum. Animals were kept for up to 5d p.i. and levels of QS signals, bacterial population as well as inflammatory parameters were determined.

PA induced loss of cell viability was partially prevented by sub-minimum inhibitory concentrations of TOB, allowing survival of infected cells. Treatment with QSI alone did not support cell survival, but combination of QSI and TOB improved cell viability compared to TOB alone, demonstrating additive antibiotic efficacy. It also stimulated IL-8 secretion, an early biomarker for neutrophil influx contributing to bacterial clearance. *In vivo* infection with alginate-supplemented PA became persistent in murine lungs after 48h and quantification of QS signals from *in vivo* samples was successfully established.

This study indicates that the combination of QSI with TOB is a promising new approach to combat persistent, biofilm-associated PA infections. This emphasizes the relevance of the QS system as valuable target for novel treatment strategies. The murine infection model seems suitable for the evaluation of *in vivo* target engagement of QS-targeting pathoblockers.

STABLE-ISOTOPE ASSISTED METABOLOMICS *IN VIVO*: CAN STABLE-ISOTOPES INCREASE THE RESOLUTION OF INDIVIDUALIZED METABOLOMICS?

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Human metabolism is a stringently regulated process and metabolic homeostasis is sensitively linked to many endogenous and exogenous factors. Already minor off-tunes in this interactions can cause disease or metabolic malfunction in the human body. On the other hand, diseases like infections might impair metabolic homeostasis and change the metabolic state of an individual. The metabolic state of an individual is imprinted in the combination of the absolute levels of all metabolites and can be accessed by metabolomics techniques. While metabolomics provides an accurate fingerprint of the current state of a biological system, it is only of a static nature. On the other hand metabolic turn-over rates or metabolic fluxes provide dynamic information on the flow of metabolites through metabolism and thus extend metabolomics by a dynamic dimension. However, metabolic fluxes can not be measured directly but need to be inferred from stable-isotope labeling experiments. For this purpose, a stable-isotope labeled pre- cursor is supplied and metabolism converts and incorporates the tracers isotopes into downstream pathway metabolites, thereby generating specific enrichment patterns are representative of underlying metabolic fluxes. Here we demonstrate how systems approaches can be applied to quantitatively monitor these metabolic processes for individual persons. We established a sensitive procedure to accurately profile glucose fluxes in humans based on oral ingestion of a 13C labeled glucose tracer in combination with dried blood spot (DBS) sampling and mass spectrometry. The application of a specific tailored ODE based metabolic model reveals quantitative and robust values for glucose production (GP) and gluconeogenesis (GNG) for each individual. We are currently ex- tending this system to a non-targeted level to determine individual and condition specific metabolic flux fingerprints.

19 IMMUNE MONITORING-GUIDED TREATMENT OF A PEDIATRIC PATIENT WITH SEQUENTIAL GVHD, ACUTE REJECTION AND CMV INFECTION FOLLOWING LUNG TRANSPLANTATION

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Background: A 17 year old patient with cystic fibrosis (HLA-A11+, CMV-) underwent bilateral sequential lung transplantation (donor: HLA-A32+, CMV+). Immunosuppression (IS) consisted of Tacrolimus, MMF and Prednisone. After an uneventful postoperative course of 3 months, he developed histology-proven cutaneous GvHD that was treated successfully by withdrawal of MMF. He developed acute rejection treated by a steroid pulse. While lung function returned to normal, the patient developed CMV infection despite valganciclovir prophylaxis. Methods: Frequencies of HLA-A32+ donor lymphocytes were measured by FACS. ELISpots were performed to detect allospecific and CMV-specific T cells. HLA-A2/NLV-pentamer staining was used to detect frequencies of CMV-specific CD8+ CTL.
Results: With development of skin GvHD, frequencies of 3-4% HLA-A32+ donor CD4+,CD8+ T cells, 5-8% B cells and 1-4% NK cells were detected for 2 weeks. Donor T, NK cells declined after MMF withdrawal; B cell frequencies remained stable. Simultaneously to improvement of GVHD, acute rejection developed, accompanied by a significant increase in frequency of allo-A32-specific CD8+ T cells within one week, which declined upon steroid pulse. Allo-HLA-A11-restricted T cells, responsible for GvHD, were found only in a low frequency. With serological detection of CMV, the frequency of HLA-A2/NLV specific CD8+ CTL increased and remained stable for four months. CMV infection disappeared with the emergence of CMV-specific CTL. Plasma levels of sCD25, IFN-g, IL-17 responded to IS alterations, to pulsed steroids with a transient drop.
Conclusions: Using specific immune monitoring tools, we could confirm clinical diagnoses of the patient. Frequencies of allo- or virus specific T cells, donor lymphocytes and plasma cytokine levels followed the clinical course of GVHD, followed by rejection followed by CMV infection. The modification of IS by using immune monitoring information resulted in a full recovery of the patient who is still asymptomatic several months after these complications.

CONTROL OF *CLOSTRIDIUM DIFFICILE*–ASSOCIATED DISEASE (CDAD) IN PATIENTS WITH PROSTHETIC JOINT INFECTIONS BY INDIVIDUALIZED ANTIBIOTIC STEWARDSHIP

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Antibacterial chemotherapy exposes patients to an enhanced risk to develop *Clostridium difficile*-associated disease (CDAD). Here we describe a cluster of 26 patients suffering from prosthetic joint infections who developed hospital-acquired CDAD. The majority of infections (n = 16) were caused by the hypervirulent *C. difficile* ribotype 027. Most *C. difficile* R027 isolates were highly resistant to levofloxacin and clindamycin and, remarkably, also to rifampicin, which are all routinely used to treat prosthetic joint infections caused by staphylococci. Due to the rifampicin resistance, rifampicin failed to counteract the clindamycin and levofloxacin enhanced dysbiosis and enhanced risk to develop CDAD. After recognition of the CDAD cluster, a bundle of infection control measures including strict isolation of CDAD patients, intensified cleaning and sporicidal disinfection was implemented together with the recommendation to avoid CDAD high risk antimicrobials such as fluorquinolones, clindamycin, and 3rd generation cephalosporins. Despite prompt implementation of infection control measures the enhanced rate of CDAD was reduced only by introducing an individualized antibiotic stewardship program. In this indABS program antibiotic treatment was taillored for every patient including avoidance of high risk antimicrobials, dosing of antibiotics, route of administration and length of therapy. This is to our knowledge the first report of an enhanced rate of CDAD caused by rifampicin-resistant *C. difficile* R027 among patients suffering form prosthetic joint infections. The successful reduction of the CDAD rate demonstrates the importance of individualized antibiotic stewardship programs in this difficult to treat group of patients.

21 CHARACTERIZATION OF HUMAN T CELL SUBSETS BY EPIGENETIC MARKER REGIONS

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Adoptive transfer of genetically engineered T cells, antigen-specific T cells or regulatory T cells (Tregs) has become a promising therapeutic approach to cure diverse life-threatening diseases. These personalized medicine approaches aim to either push inflammatory reactions or induce tolerance. Qualitative aspects, like expression of T cell subset-specific lineage factors or effector molecules seem to be important for the success of the treatment. Characterization of T cells by quantifying RNA or protein expression levels bears the risk of misinterpretation due to temporary up- or down-regulation of marker genes at the time point of measurement. In contrast, measuring DNA methylation patterns is more reliable because differentiated T cell populations contain specific marker regions that are permanently modified and less sensitive to transient changes.

For human Tregs we already could identify a region within the locus of the master transcription factor FOXP3, called Treg-specific demethylated region (TSDR), which can be used in the clinic to identify stable Tregs. Recently, we were able to detect a higher number of stable Tregs in patients suffering from severe atopic dermatitis (SCORAD > 50) by measuring the TSDR methylation rate. In another study, we measured the TSDR methylation rate of CD4+ T cells from elderly individuals participating in an influenza vaccination trial. Interestingly, preliminary results revealed a trend that higher numbers of stable Tregs before vaccination correlate with non- responsiveness to vaccination, a serious problem for the elderly. Further DNA methylation profiling studies on human CD4+ T helper cell subsets (Th1, Th17, ncTh1) were already performed to identify novel epigenetic biomarkers for their unequivocal characterization and quantification. In addition to expected regions linked to lineage specific factors for Th1 or Th17 cells like T-bet, IFN-g, RORgt or IL-17A, additional uniquely demethylated regions were identified allowing the development of epigenetic signatures for the characterization of T helper cell subsets in the clinical practice.

INDIVIDUALIZED ANTIBIOTIC TREATMENT FOR URINARY TRACT INFECTIONS AT POINT OF CARE – FAST-TRACK-DETECTION OF *ESCHERICHIA COLI* AND ITS RESISTANCE BY A GENOME- TAILORED LAMP-PANEL

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Empirical prescriptions of antibiotics for urinary tract infections (UTI) are becoming problematic since uropathogens like *E.coli* increasingly exhibit resistance against classical antibiotics like ampicillin/sulbactam, trimethoprim/sulfamethoxazole and ciprofloxacin. These antibiotics face significant resistance rates in many clinical settings, which leads to the use of more and more broad spectrum antibiotics in empirical situations. Broad spectrum antibiotics generally exhibit larger collateral damage then narrow spectrum antibiotics.
The current “Gold Standard” of microbiological culture takes two days, which often delays urological interventions until after results are available, and leads to prescription of broad-spectrum antibiotics (BSA) already in the prophylactic setting. A fast point of care test for antibiotic resistance is highly desirable to aid clinicians in selecting the appropriate antibiotic at first patient visits, as it may allow physicians to immediately use a small spectrum antibiotic when applicable, thus diminishing the use of unnecessary BSA.
We are developing such a test on the basis of a LAMP (loop-mediated isothermal amplification) panel which detects *E.coli* (the most common uropathogen) and its most relevant resistances.
To define a locally suited genetic detection panel, we collected all *E.coli* strains of urological patients over 14 months, regardless of phenotypic resistance, from in- and outpatients of all ages and both genders. We sequenced these strains using Next-Generation-Sequencing and analyzed their genomes in our assembly, annotation and analysis pipeline to determine suitable targets for molecular detection of resistance determinants. We then used this data to construct a detection panel for *E.coli* and its common relevant resistance genes.
Determination of urinary bacterial loads is essential for diagnosis of UTIs. Using our test principle, we can estimate this by considering time-to-positivity of the amplification curves of the LAMP reaction.
We have evaluated our urine point of care test in a pilot field study on fresh urine samples of patients of our university urological department. We demonstrate that our system can a) detect and semi-quantify *E.coli* in prospective urine samples and b) forecast relevant antibiotic resistances within 1 hour after urine sampling, at the point of care, thus promising to be a valuable tool for individualized infection medicine, rapidly at the point of care.

23 DYNAMIC ALTERATIONS IN GUT MICROBIOTA COMPOSITION IN PATIENTS UNDERGOING ALLOGENEIC PERIPHERAL BLOOD STEM CELL TRANSPLANTATION

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Allogeneic peripheral blood stem cell transplantation (allo-PBSCT) is a therapy to treat neoplastic diseases. Patients undergoing an allo-PBSCT experience severe perturbation in the immune system and in the gastrointestinal mucosa. It has been shown that low gut microbiota diversity correlates with transplant-related mortality and increases the risk of bacteremia [1,2]. While previous studies have been performed in cohorts from the USA, little is known about the dynamics of microbiota composition in European patients undergoing different transplantation protocols. Here we longitudinally characterized the dynamic composition of the gut microbiota in a cohort of more than fifty German patients (n=55) undergoing allo-PBSCT.
Using multivariate statistics and unsupervised clustering methods, we identified three cluster-states based on diversity and microbial composition of the samples. Cluster ***High*** diversity (Inv. Simpson index >4), corresponded to the group of samples before allo-PBSCT and mostly after the six week of the treatment. ***Intermediate*** (Inv. Simpson index = 2-4), which mainly includes samples after the third week and ***Low*** diversity individuals (Inv. Simpson index <2), which largely included the samples early after transplantation. We identify distinct bacterial biomarkers for these clusters, and their dynamics during the therapy. Our data support the observation proposed in the American cohort and provide novel microbial signatures that can be used in the design of the antibiotic protocol and improvement of the PBSCT therapy.

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NEXT GENERATION SEQUENCING TO CHARACTERIZE THE HUMAN CYTOMEGALOVIRUS GENOME DIVERSITY DIRECTLY FROM CLINICAL SAMPLES

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The human cytomegalovirus (HCMV) remains a frequent cause of complications in transplant recipients or congenitally infected children. Its genome encodes at least 167 open reading frames (ORFs) and contains several hypervariable genes. Interstrain recombination, reactivation of latent virus, as well as multiple strain or re-infections additionally contribute to the genetic diversity of HCMV in an individual host. However, the impact of this diversity on pathogenesis and clinical outcome of HCMV infections is unclear. The advent of next-generation sequencing (NGS) allows comprehensive insights across the entire HCMV genome.

We use a target capture protocol for high-throughput sequencing of HCMV populations directly from a variety of clinical specimens. Both, viral consensus genome and variant analyses are feasible and allow to characterize the HCMV population composition in single samples, as well as in multiple samples collected longitudinally or from different anatomical compartments of individual patients.

Our data show that multiple infections involving two or more HCMV strains are relatively common especially in transplant recipients, and (in a hematopoietic stem cell transplant cohort) seem to be associated with an earlier peak of HCMV-antigenemia after transplantation. In a number of individuals a switch of the dominant HCMV population over time (and more rarely among different compartments) has been observed, suggesting that prolonged HCMVemia, viral tissue tropism or other features might be linked to different viral populations with potentially different biological properties. Finally, antiviral resistance mutations, an important clinical issue, could be tracked longitudinally and among compartments using our NGS protocol.

NGS will play a significant role to investigate the intra-host composition of HCMV populations or to identify variants such as resistance mutations or certain biomarkers that could be used for risk stratification and individual tailoring of preventive and therapeutic strategies.

25 ESTABLISHMENT OF A SINGLE-POINT ASSAY TO EVALUATE THE RELATIONSHIP OF MOLECULAR STRUCTURE AND ANTIVIRAL ACTIVITY OF CATIONIC AMPHIPHILIC DRUGS

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Enveloped virus families *Filovidirae*, *Arenaviridae*, *Rhabdoviridae*, *Coronaviridae*, *Togaviridae*, *Flaviviridae* and *Bunyaviridae* enter host cells via endosomal trafficking and low pH-dependent early or late membrane fusion. The lack of effective antiviral treatment or vaccination makes novel antiviral drug development necessary in order to alleviate morbidity and mortality in epidemics. Cationic amphiphilic drugs (CADs) were found to have broad antiviral properties but evoke also concerns about adverse effects in host cells, such as drug-induced phospholipidosis (DIPL). Nevertheless, the antiviral mechanism of action and structure-function relationship of CADs is yet unclear. In this project, we aim to assess and compare the antiviral activities of CADs in a single-point approach and analyze which structural determinants enhance antiviral potential.

Due to CAD-sensitivity and its high titer, Marburg virus (MARV) lentiviral particles were determined as best-suited pseudovirion for this drug-screening system. For the prototypical CAD amiodarone, IC50 = 5 µM and CC50 = 28.7 µM were determined in human endothelium/lung hybrid cells (EAhy 926). With these defined conditions a single-point assay was established with 5 µM as an optimal concentration to apply to all CADs.

The single-point testing of a panel of 47 CADs with various physico-chemical properties revealed clear differences between the CAD's ability to reduce viral transduction. The strongest antiviral activities were achieved with Dronedarone, Triparanol and Quinacrine with residual viral entry of 16 %, 18 % and 23 %, respectively. Simultaneously, the CAD's cytotoxicity and DIPL effects were evaluated. Comparison of antiviral activities and the CAD's physico-chemical properties showed a positive correlation between antiviral activity and hydrophobicity, indicating logP>4 for strong antiviral CADs. In order to underline our findings, similar compounds to strong CADs will be identified and tested in several approaches.

ON THE WAY TO UNDERSTANDING METABOLIC ASPECTS OF HOST-PATHOGEN INTERACTIONS DURING *CLOSTRIDIUM DIFFICILE* INFECTION

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C. difficile had been identified as causative agent of pseudomembranous colitis (PMC) in the 1970s. It causes antibiotic-associated intestinal infections attributed by symptoms reaching from mild diarrhoea to toxic megacolon. The major virulence factors of *C. difficile* are Toxin A (TcdA) and Toxin B (TcdB) which present a cytopathic as well as cytotoxic effect. The cytopahtic effect is mediated by intracellular glucosylation of Rho GTPases and consequently depolymerizing actin cables which leads to the loss of cell shape. The cytotoxic effect is induced by generation of reactive oxygen species (ROS) leading to early cell death. Intriguingly, mitochondrial metabolism seems to play a crucial role in the cytotoxicity of these enterotoxins, because TcdA partly localizes into mitochondria already 5 min after toxin treatment. This is associated with cytochrom c release, depletion of ATP levels and membrane potential. To better understand metabolic aspects of the infection and the role of the enterotoxins, we will profile intracellular metabolic fluxes in macrophages and epithelial cells with stable-isotope labeling. First results indicate a strong pro-inflammatory activation of mammalian macrophages by TcdA, while TcdB exerts other and less inflammatory metabolic effects. To decipher the role of mitochondrial metabolism during the infection we established a strategy to get an easy and quick access to functional mitochondria by selective permeabilization of the cytosolic membrane. Using this setup, we will study mitochondria of cells that have been previously exposed to the toxins. In combination with stable isotope-labeling and bioinformatics data processing we will reveal how and to which extend *C. difficile* toxins rewire mitochondrial and whole cell metabolism. Information on these metabolic switches has the potential to reveal new intervention points.

27 A FUNCTIONAL *EX VIVO* CO-CULTURE ASSAY FOR DYNAMIC CHARACTERIZATION OF CYTOLYTIC IMMUNE RESPONSES AGAINST VIRUS INFECTED HUMAN CELLS

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Cell-mediated cytolytic immune response is indispensable for the function of the immune system in humans and other complex organism, with its great potential for therapeutic approaches indicated by the remarkable effectiveness of Adoptive Cell Transfer (ACT) therapies using CD8+ cytotoxic T lymphocytes (CTL) against certain cancers and viral infections. While *in vivo* murine models along with *ex vivo* or *in vitro* assays with primary cells or cell lines, which relay on quantification of chromium release from lysed cells and/or virus titration, are widely used for functional assessment of cytolytic cells, these may be limited by their relevance to human viruses and more importantly, they only provide indirect snapshots that may mask intricate dynamic processes.

We have established a co-culture assay using *ex vivo* human cytomegalovirus (HCMV) specific CD8+ T cells together with HLA haplotype-matched target cells, infected with reporter HCMVs expressing non- fusion fluorescent reporters under the control of endogenous promotors of immediate early (IE), early (E) or late phase HCMV genes in equimolar ratios. Using confocal live cell imaging, we collect data at high temporal resolutions for up to a week and use semi-automated software analysis to quantify viral gene expression, secondary spread of the infection, target killing and other parameters, at the single-cell or population level. For instance, *ex vivo* CD8+ T cells specific for HLA-A2 restricted epitope NLVPMVATV from HCMV pp65 are effective controllers of TB40E infection in HLA-A2 human fibroblasts even at low effector to target ratios.

The flexibility and robustness of the assay allows for its easy adaptation to other questions, where various effector and target cells, reporter viruses, as well as cell tracker reagents, extracellular antibodies and viability stains can be used. This provides for a versatile platform for detailed elucidation of dynamic processes during viral infections.

SEROLOGICAL DIAGNOSTICS: LINKING SELF-SAMPLING WITH DIFFERENTIAL SEROLOGY

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Differential Serology identifies discriminatory patterns in the immune response of individuals to distinguish vaccinated from infected individuals with minimal sample consumption and the potential for automated high-throughput multiplex application.
The tool allows the generation of data from population-based studies on vaccination coverage and seroprevalence to translate into personalized risk stratifications. The provided information of the disease burden and vaccine efficacy/effectiveness can be seamless integrated into the decision-making process for individualized prevention strategies as well as tailored therapy recommendations. The ability of the method to identify the individual vaccination-infection status of a patient for multiple vaccine preventable diseases simultaneously helps to further adjust tailored prevention or treatment strategies. We aim at reforming the currently rather crude vaccination recommendation basing them not only on rough age groups but on stratified biomarker analysis.
We have made progress in this regards by providing a proof of concept project with the development of a differential serology targeting infections with the hepatitis A virus. Currently, we are verifying biomarkers for hepatitis B and E to formulate a complete viral hepatitides panel based on the existing differential serology for hepatitis A.

29 AN *IN SILICO* ANALYSIS OF PATIENT-ADAPTIVE INTERLEUKIN-2 THERAPY IN AUTOIMMUNE AND INFLAMMATORY DISEASES

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Regulatory T cells (Tregs) are suppressor cells that control self-reactive and excessive effector T cells (Tconvs) responses. Breakdown of the balance between Tregs and Tconvs is the hallmark feature of autoimmune and inflammatory diseases. Due to the positive dependency of both populations on Interleukin-2 (IL-2), it is a subtle leverage to restore the healthy immune balance. By employing a mechanistic mathematical model, we studied the IL-2 therapy for stabilizing Treg population and restricting inflammatory Tconv response. We introduced an adaptive control strategy to design the minimal IL-2 dosage. This adaptive strategy allows for an individualized therapy based on the feedback of immune kinetics of patients. Our *in silico* results suggest that a minimal Treg population is required to restrict the transient positive effect of IL-2 injections on effector Tconv response. The combination of IL-2 and adoptive Treg transfer therapies is able to limit this side-effect in our simulations.

MICROBIOME SIGNATURES IN INDIVIDUALS AT RISK FOR RHEUMATOID ARTHRITIS

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Rheumatoid Arthritis (RA) is an autoimmune disease and is currently thought to be triggered by a combination of genetic and environmental factors. Specifically, a previous cohort study identified a distinct microbiome signature, i.e. an overabundance of *Prevotella copri*, in newly diagnosed RA patients [1]. In support of a role of *P. copri* in the development of RA, sequence homologies between RA-specific autoantigens and proteins of *P. copri* have been reported [2]. Yet, whether alterations in the microbiota precede the development of autoimmunity or rather are consequences of autoimmunity in the host remains to be studied.
Here, we have characterized microbiota composition in a cohort of human individuals displaying pre- clinical phases, of RA (autoantibodies, but no active disease, preRA) and first degree relatives of patients (RA-FDR) without autoantibodies as controls using 16S rRNA gene sequencing. Statistical analyses of community structures were performed [3].
Our analysis demonstrated that the microbiota of preRA individuals was significantly altered compared to RA-FDR, with a relative overabundance of specific bacteria, particularly an enrichment of members of the genus Prevotella. PICRUSt was used to predict the functional profiles form the cohort and shotgun sequencing was used to obtain metagenomics species of different *Prevotella* species.
Our findings support the hypothesis of a role of *Prevotella spp* in the development of RA, which could lead to future attempts to interfere with its intestinal colonization during the preclinical stages of disease.

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31 VIRUS-SPECIFIC T CELLS FROM STEM CELL, FAMILY AND THIRD PARTY T CELL DONORS: PATIENT MONITORING, DONOR SELECTION AND GMP-COMPLIANT MANUFACTURING

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Background: Intensive immunosuppressive therapy for prevention of graft rejection and graft-versus-host disease (GvHD) and for treatment of GvHD puts the patients before and after hematopoietic stem cell transplantation (HSCT) or solid organ transplantation (SOT) at risk of opportunistic infections due to an ablated or severely compromised T-cell immune response. Among those, infection with and reactivation of endogenous herpes viruses like cytomegalovirus (CMV), Epstein-Barr virus (EBV), human herpesvirus 6 (HHV6), lytic agents such as adenovirus (ADV) as well as polyoma virus BK (BKV) are frequent and severe complications and associated with significant morbidity and mortality. The shortcomings of conventional therapies have increased the interest in antiviral T-cell transfer. The efficacy and the clinical outcome in high risk patients can be improved by a rapid recruitment of a suitable T-cell donor and an established method for fast manufacturing of antiviral T cells.
Methods: To facilitate and accelerate donor recruitment a registry (alloCELL) for unrelated donors was established. The registry currently records >2000 HLA-typed donors extensively screened for their antiviral T-cell repertoire. The alloCELL lab further established comprehensive protocols to consider clinical requirements of patients at high risk for viral infections or with failed conventional therapy. The manufacturing license was obtained for generating clinical-grade mono- and multivirus-specific T-cell products according to the German Medicinal Products Act using the IFN-γ Cytokine Capture System and CliniMACS Prodigy device. T-cell donors were defined as eligible if ≥0.03% specific IFN-γ^+ T cells are detectable. A related haploidentical or at least 3/6 HLA-A/B/DR-matched alloCELL donor is recommended, if the stem cell donor is not eligible.
Results: Antiviral T-cell frequencies in patients after stem cell or solid organ transplantation were determined routinely by ELISPOT and multimer staining. 91 out of 151 patients without detectable antiviral T-cells and without sustainable response to conventional antiviral treatment were assigned to receive virus-specific T-cell products. For those 141 donors were tested: 49 family, 27 stem cell, 65 alloCELL donors, from whom 82 clinical-grade antiviral T-cell products were generated.
52 (62%) patients received T-cell products specific for CMV, ADV, EBV and BKV alone or in combination from related (17, 21%) or alloCELL (35, 43%) third-party donors. For patients in need of an unrelated third party donor we were able to find a suitable donor and provide the clinical grade T-cell product in less than 1.5 weeks after request with an HLA compatibility ≥5/10. T cells applied were monitored in patient blood to determine frequency, chimerism and T-cell receptor repertoire. Patients received antiviral donor T-cells without significant side effects and in 80% antiviral T cells became detectable after T-cell transfer.
Conclusion: Success of antiviral T-cell transfer benefits from (i) accurate monitoring of viral load and antiviral T-cell frequencies in patients, (ii) early and fast selection of suitable T-cell donors. Our data support clinical safety and efficacy of third-party antiviral T cells.

GENETIC VARIANTS IN HEPATITIS C VIRUS ENTRY FACTOR GENES INFLUENCE HCV INFECTIVITY

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The clinical course of HCV infection shows remarkable inter-individual variation in terms of susceptibility and disease progression. Host genetic variability may affect early infection events including the virus entry into host cells. HCV entry requires the cell surface tetraspanin CD81, scavenger receptor class B1 (SR-B1), Claudin (CLDN1) and Occludin (OCLN). Several coding and non-coding variants in CD81 and SCARB1 (gene encoding for SR-B1) and their influence on HCV susceptibility have been studied. Interestingly the impact of the regions outside the large extracellular loop (LEL) of CD81 and the molecular biology of the SCARB1 variants remained largely elusive. Our aim was to decipher the consequences of coding SCARB1 variants and CD81 variations in non-LEL regions on the HCV replication cycle *in vitro*.

We expressed SR-B1 and CD81 variants in hepatoma cell lines lacking the respective factor or in stem cell derived hepatocytes. SCARB1 coding variants rs397514572 and rs187831231 were expressed at the cell surface but appeared impaired in lipid and HCV glycoprotein E2 binding and – most importantly – failed to rescue HCV infectivity in hepatoma cells where endogenous expression had been ablated. For CD81 we demonstrated that chimeras for the CD81 LEL with non-LEL regions of distantly related tetraspanins failed to support HCV entry, implying a role of those regions in HCV entry. Furthermore, by exchanging the cholesterol coordinating E219 residue in transmembrane domain 4 to a non-cholesterol binding residue, we observed reduced HCV infectivity. In summary the data underlines that single residue exchanges in HCV entry factors as well as non-conding variants can impact HCV susceptibility and disease parameters. This work holds the promise of a better understanding of inter-individual differences in HCV susceptibility and disease outcome.

33 HUMAN MUCUS ENVIRONMENT DIFFERENTIALLY AFFECTS *PSEUDOMONAS AERUGINOSA* SUSCEPTIBILITY TOWARDS ANTIBIOTIC TREATMENT

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Background:
Cystic fibrosis (CF) is associated with altered mucus secretion which impairs mucociliary lung clearance. This condition facilitates the acquisition of biofilm-associated bacterial lung infections, in many cases by *Pseudomonas aeruginosa*. Biofilm formation is associated with increased antibiotic tolerance leading to an aggravated treatment situation. Common *in vitro* assays to test antibiotic efficacy of biofilms do not consider mucus as natural environment for biofilms in the lung. In the presented study we investigated the impact of human mucus on antibiotic efficacy versus *P. aeruginosa* biofilms.
Materials/methods:
Human tracheal mucus was collected from patients undergoing elective surgeries with no association to any lung disease. Mucus was characterized, freeze-dried and rehydrated prior to infection experiments. *P. aeruginosa* (strain PAO1) biofilms were grown in presence or absence of mucus for 24h at 37°C under static conditions. Biofilms were treated with tobramycin or colistin at doses of 100x, 300x and 900x MIC. Bacterial load was determined by dilution plating and enumeration of colony- forming-units. Biofilm structure was visualized via confocal microscopy. Antibiotic penetration was assessed via diffusion studies and following HPLC measurement.
Results:
In presence of human mucus, *P. aeruginosa* biofilms showed significantly increased antibiotic tolerance towards treatment with tobramycin, but not colistin. Diffusion studies showed that tobramycin penetration was retarded and decreased in presence of mucus, however, no differences of colistin diffusion were observed. Confocal images revealed a denser biofilm structure and a higher number of fluorescent signals indicating a higher bacterial load in presence of mucus.
Conclusions:
Human mucus environment alters *P. aeruginosa* biofilm formation resulting in a more compact biomass and higher bacterial numbers. This consequently leads to altered susceptibility towards antibiotic treatment with tobramycin but not colistin, depending on the differential impact on antibiotic diffusion. This model could serve as a valuable tool for studying the impact of mucus in chronic *P. aeruginosa* infections and efficacy testing of novel anti-infective compounds.

TRANSCRIPTOMIC ANALYSES REVEAL ANTI-VIRAL RESPONSES OF EPITHELIAL CELLS AND MULTIPLE IMMUNE CELL TYPES IN HRV INFECTED HUMAN LUNG TISSUE

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Human rhinovirus (HRV) is a main cause of airway infections and a major risk factor of exacerbations in asthma and COPD. Investigation of HRV pathogenesis has been hampered by the lack of complex *in vitro* models that closely represent the human disease. The aim of the study was to characterize the immune response of viable human lung tissue to *ex vivo* HRV infection using *Precision-Cut Lung Slices* (PCLS).

Human PCLS containing airways were inoculated with HRV1B, UV-inactivated HRV, medium or HRV in the presence of 3C protease inhibitor Rupintrivir. At day 1 and day 3 post infection (p.i.) tissue vitality, viral load and cytokine release were measured and transcriptomic analyses upon RNA isolation from PCLS were performed.

HRV infection of human PCLS induced no strong cytopathic effect as indicated by intact tissue viability. The transcriptomic analyses revealed that HRV infection of PCLS induced 5977 and 4322 gene expression changes at day 1 or day 3 p.i., respectively. These gene signatures were indicative of interferon signalling, epithelial cell differentiation, lymphocyte regulation, antigen presentation and NK cell cytotoxicity. Rupintrivir downregulated about one third of the HRV upregulated genes. These data were confirmed by increased protein levels of pro- inflammatory and anti-viral cytokines induced by HRV, e.g. TNF-α and IFNα2a, which were also diminished by Rupintrivir.

In conclusion, *ex vivo* infection of human lung tissue with HRV induced a strong antiviral and pro-inflammatory immune response. The observed gene expression profile revealed involvement of epithelial but also multiple immune cells. This enables us to study HRV induced immune responses in the human lung microenvironment.

35 INDIVIDUALIZED HCMV-GB-CAR T CELLS TO TREAT HCMV REACTIVATION IN POST-TRANSPLANT PATIENTS

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Introduction: Human cytomegalovirus (HCMV) can be controlled by immune competent heathy subjects but remains latently through several mechanisms of immune suppression and escape. Reactivated infection with HCMV is associated with poor outcome in immune compromised hosts after stem cell transplantation (SCT). Adoptive transfer of virus-specific T cells in an SCT-setting has proven efficacious, but the approach is problematic when seropositive donors are not available, such as in the case of cord blood (CB)-SCT. Hence, we designed T cells expressing HCMV-specific chimeric antigen receptors (CARs) from HCMV seronegative donors to control viral lytic reactivation. The HCMV-glycoprotein B (gB) was used as target, because it is a highly conserved surface-bound protein abundantly expressed on cells hosting lytic replication. We further tested if a professional antigen presenting cell expressing gB would provide optimal homeostatic and antigenic stimuli to maintain gB-CAR T cells active.
Methods: gB-CARs were constructed by fusion of single-chain variable fragments of a highly affine gB-specific human mAB (SM5-1) to CAR-backbones comprising CD28/CD3ζ and 4-1BB/CD3ζ domains. Transduction of human T cells from PBMC and CB with γ-retroviral vectors yielded 60- 95% CAR-expression.
Results: For *in vitro* potency assays gB-CAR T cells we used mesenchymal stem cells (MSCs) permissive to HCMV-infection. MSCs were infected at multiplicity of infection (MOI) of 10-2 with the HCMV-strain TB40 expressing a secretable Gaussia luciferase (HCMV-gLuc). Both CD28/CD3ζ and 4-1BB/CD3ζ-containing gB-CAR T cells effectively killed infected MSCs, assessed by lower detection of gLuc-luminescence and death of target cells by flow cytometry. gB-CAR T cells co- cultured with HCMV-infected MSCs proliferated and secreted IFNγ. Neither CD19-CAR T cells nor uninfected MSCs reproduced these effects. A serial killing assay demonstrated persistent killing with less pronounced exhaustion (measured by PD-1 expression) for the 4-1BB/CD3ζ-containing gB- CAR T. Using Nod.Rag mice transplanted with human hematopoietic stem cells infected with HCMV-gLuc and treated with G-CSF for reactivation, we could follow bio-distribution of the infection by optical imaging analyses. Pilot results indicated gB-CAR T cells *in vivo* reduced the levels of HCMV infection measured by PCR and optical imaging.
Conclusions: gB-CAR T cells recognize HCMV-infected cells *in vitro* and generate cytotoxic effects. The effectivity of gB-CAR T cells to control HCMV infection *in vivo* in humanized mice is currently being further investigated. Since HCMV-specific CARs can reprogram naïve or memory T cells from different sources (CMV- PBMC donors cord blood) to react against HCMV in an HLA-independent manner this approach would vastly facilitate the generation of adoptive T cells for patients in need.

INDIVIDUALIZED PHAGE THERAPY IN THE CLINICAL SETTING

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Emergence of multidrug- and even pandrug-resistant bacteria has led to the necessity of developing new antibacterial drugs. One of the viable alternatives to known antibiotic therapy is the application of lytic bacteriophages. Bacteriophages (or phages) are viruses that are able to infect bacterial cells only. Lytic bacteriophages may be used as antibacterial drugs, as they cause an active infection, which leads to bacterial cell lysis.
Phages have relatively narrow host range activity in comparison to conventional antibiotics. Each bacteriophage is typically active against only some bacterial strains or isolates within certain species. Wide host range activity of phage-based products is often achieved through a mixture of different strains in a phage cocktail. However, phage cocktails need to be permanently updated because of the changes of circulating isolates and the development of phage resistance.
The efficacy of phage therapy has been proved for decades in the following countries where bacteriophage cocktails are registered as drugs or medical products: Russia, Georgia, Kazakhstan and Slovakia. But the rapid change of epidemiologically relevant bacterial isolates in the clinical setting leads to inefficiency of this approach against healthcare-associated bacterial infections.
Our aim is to establish an individualized phage therapy based on a permanently updated collection of well-characterized lytic bacteriophages. We have developed standardized methods for the manufacture of individualized phage preparations. However, such an individualized phage therapy is currently only possible as an *ultima ratio* therapy according to § 37 of the Declaration of Helsinki in a majority of legislations. Further development of methods to expand the cohorts of patients suitable for receiving individualized phage therapy in earlier stages of a bacterial infection is ongoing.

37 INVESTIGATION OF HETEROZYGOUS NFKB1 VARIANTS IN A COMMON VARIABLE IMMUNODEFICIENCY COHORT

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Nuclear factor kappa B (NF-κB1) is a transcription factor, which is sequestered within the cytoplasm of every cell. During activation of the canonical NF-κB pathway, NF-κB1 translocates to the nucleus and binds to the promotors of its target genes. NF-κB1 has been linked with a diversity of diseases, including asthma, AIDS, diabetes and cancer. More recently, it has been shown that *NFKB1* mutations could lead to haploinsufficiency of the active subunit p50 and therefore could cause the CVID phenotype. CVID as a syndrome comprises a heterogeneous group of molecular diseases, characterized by a significant hypogammaglobulinemia of unknown cause.
Genomic DNA for targeted-NGS was isolated from whole blood. Detected mutations were validated by Sanger sequencing. PBMCs were isolated by density gradient centrifugation and stimulated with PMA plus ionomycin and analyzed using immunoblotting with antibodies against NF-κB1 p105 and p50. Tubulin or ß-actin was included as a loading control.
In our study we identified in seven patients six novel heterozygous mutations in *NFKB1* by targeted-NGS. Among those, one frameshift deletion, three single base-pair insertions, one missense and one splice site mutation. *NFKB1* mutations occur in our CVID cohort with a prevalence of 1:30. In all affected members of three families, their mutations lead to a reduction of the active NF-κB1 subunit p50. Nevertheless, the mutations segregate with incomplete penetrance in families.
Mutations in *NFKB1* could lead to reduced levels of p50. Due to the incomplete segregation of penetrance, other causes, like epigenetic patterns or intestinal microbiome may promote the onset of disease.

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BAICD GENE ABUNDANCE IS NEGATIVELY CORRELATED WITH *CLOSTRIDIUM DIFFICILE* INFECTION

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Background: *Clostridium difficile* infection (CDI) is a major cause of hospital- acquired diarrhea. Preconditions are colonization with *C. difficile*, but also a breakdown of the colonization resistance, of which secondary bile acids are believed to constitute a decisive component. 7α-dehydroxylation is one of the key steps in primary to secondary bile acid transformation, and required genes have been located in a single bile acid inducible (bai) operon in *C. scindens* as well as in *C. hiranonis*. The prevalence of these species in human fecal samples – particularly with regard to samples tested positive for *C. difficile* – and in the setting of fecal microbiota transfer (FMT) in CDI patients has not been studied.
Aim: To analyze *baiCD* gene abundance in *C. difficile* positive and negative fecal samples.
Material & Methods: A species-specific qPCR for detection of *baiCD* in *C. scindens* and *C. hiranonis* was established. Fecal samples of patients with CDI, toxigenic *C. difficile* colonization (TCD), non-toxigenic *C. difficile* colonization (NTCD), of *C. difficile* negative (NC) patients, and of two patients before and after fecal microbiota transfer (FMT) for recurrent CDI (rCDI) were tested for the presence of the *baiCD* gene cluster.
Results: In NC samples, the prevalence of the *baiCD* gene cluster was significantly higher than in CDI samples, with 72.5% (100/138) vs. 35.9% (23/64; $p<0.0001$), respectively. No differences were seen between NC compared with NTCD samples or TCD samples. Both rCDI patients were *baiCD* negative at baseline, but one patient turned positive after successful FMT from a *baiCD* positive donor. *BaiCD*/16S rDNA ratio calculation showed no significant difference between the relative abundances in both groups (NC vs. CDI; $p=0.3244$). The median of the NC group was 0.006% (IQR 0.0005%-0.376%) and for the CDI group 0.009% (IQR 0.0002%-7.8%). In addition, stool consistency did not measurably influence the relative abundance of bacterial species in this context of complex microbial communities.
Conclusion: *BaiCD* gene positive species are reduced in fecal samples of patients with *C. difficile* infection as compared to asymptomatic carriers or *C. difficile* negative fecal samples. Furthermore, we present a case of *baiCD* gene positivity observed after successful fecal microbiota transfer for recurrent CDI.

39 IDENTIFICATION AND CHARACTERISATION OF HLA-A2 RESTRICTED CD8+ T CELL IMMUNE HIERARCHY AGAINST FULL LENGTH HEPATITIS E VIRUS (HEV) FOR CHRONIC HEV T CELL-BASED THERAPY DEVELOPMENT

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INTRODUCTION Hepatitis E virus (HEV) infection is often subclinical and resolves spontaneously in immunocompetent people, but it could lead to chronic hepatitis in immunosuppressed patients. Ribavirin is the only drug available for chronic HEV hitherto; however, cases of Ribavirin-resistant have occurred. We aim to identify immune hierarchy of CD8+ T cell epitopes towards full length HEV and characterize the immune functionality, as the basis to develop an alternative chronic HEV therapy.
METHOD CD8+T cells from acute HEV patients (n=13) and healthy cohort (n=9), all HLA-A2 positive, were expanded *in vitro* using 15-mer HEV genotype 3 overlapping peptides. HEV-specific T cells was identified by dextramers (epitopes are predicted *in silico*) and their immune functionality by intracellular cytokines staining. HEV-specific T cell receptor (TCR) repertoire sequencing results are used to design TCR redirection assay, followed by a killing assay to examine target specificity.
RESULTS HEV-specific responses are found across ORF1 and ORF2 of HEV genome, notably in HEV patients. Among all the responders, an epitope from pool 6 (RNA helicase region) was dominant in HEV patients; while another from pool 8 (RNA-dependent RNA polymerase, RdRp region) was found in both cohorts, thus selected for further characterization. In TCR redirection assay, new donor's lymphocytes undergone redirection were able to express TCR that bind the same dextramer, and recognize its cognate peptide presented by T2 cells with significant IFNγ and TNFα production within 7 hours of co-culturing. In addition, higher cell death in peptide-loaded T2 cells was detected after 4 hours of co-culturing. Cytokines production was also observed when redirected lymphocytes co-cultured with HEV-transfected HepG2 cells.
CONCLUSION Our project has identified a TCR targeting a dominant HEV-specific CD8+ T cell epitope, which is of substantial clinical implication for developing a potential immunotherapy targeting chronic HEV.

MICROBIOME-HOST INTERACTION IN ORAL PERI-IMPLANT PATHOLOGY AND DIAGNOSIS; A METATRANSCRIPTOME ANALYSIS

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Oral peri-implant diseases influence quality of life, systemic health, and expenditure. Infections are common (prevalence > 20 %), recurrent, can progress rapidly, and lead to implant loss. Disease results from disrupted homeostasis between the tissue and biofilm. Early diagnosis is desirable because delayed therapy is often ineffective. No single uniform definition of peri-implantitis and high interpersonal diversity are the main challenges that can be overcome by a personalized approach.

The aim of this study was to describe microbiome-host interactions in peri-implant pathology and identify biomarkers of disease. To cover different stages of disease at population and patient levels we sampled 134 implants from 53 individuals. mRNA from biofilm and crevicular fluid was sequenced yielding 2.7 billion raw reads. On average 3.5 million reads were mapped to bacterial genomes per sample.

A steady gradation in active community structure was observed across samples with imprecise borders between four main community types. Pocket depth, amount of crevicular fluid, and plaque index correlated with changes in community structure. Intraindividual diversity revealed the two potential types of long term biofilm dynamics. Communities from health and mucositis (characterized by inflamed soft tissue surrounding an implant) were generally similar with an exception of a Betaproteobacteria-rich community type observed almost exclusively in a subpopulation of mucositis samples. Community structure in peri-implantitis (characterized by peri-implant bone loss) was distinct from two other groups and characterized by replacement of Bacilli by Bacteroidia. Decreased activity of 12 *Streptococcus* spp. was associated with peri-implantitis. Canonical analysis of principal coordinates correctly predicted diagnosis for 70% samples using taxonomic data and leave-one-out approach. We expect that functional and host data, currently under investigation, will yield biomarkers that considerably improve sample allocation.

Our results enhanced the understanding of the biofilm dynamics that are crucial for peri-implant disease and revealed oral taxa that may play key roles in this process. Ultimately, best biomarkers should enable early diagnosis, identify the stage of disease and indicate the most effective therapy.

41 INFECTION OF HUMANIZED MICE WITH A HCMV STRAIN EXPRESSING GLUC: REACTIVATION ASSESSED BY NON-INVASIVE OPTICAL IMAGING IS ASSOCIATED WITH T AND B CELL IMMUNE MODULATIONS

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Introduction: Human cytomegalovirus (HCMV) infections and reactivations cause significant post- transplant complications. Although antivirals have substantially decreased the incidence of HCMV- associated disease during the early post-transplant period, they can cause significant toxicities and adverse immune effects. Unfortunately, no vaccine, HCMV-specific monoclonal antibody or cellular therapy has been licensed to date. A major obstacle for the development of new immunologic treatments is the lack of *in vivo* model systems of HCMV infection to obtain knowledge about their efficacy, potency and safety.
Methods: MRC-5 lung fibrobast cells were infected with a laboratory adapted HCMV strain expressing Gaussia luciferase driven by the IE1 promoter (TB40-IE1-GLUC [1]) and cryopreserved.. Nod.Rag.Gamma (NRG) mice transplanted with CD34+ cord blood HSCs were monitored for 15-17 weeks for reconstitution of human T and B cells. Subsequently, 106 infected MRC-5 cells were administered i.p. per mouse at week 17. At final time-points after infection, coelenterazine was infused i.v., and the total flux (p/s) and the average radiance (p/s/cm2/sr) was measured. After sacrifice, mouse tissues and viable cells were collected and cryopreserved for RT-q-PCR, IHC and multicolor flow cytometry of immature, mature and activated T and B cells from lymphatic tissues.
Results: Luminiescence signal was detected in the liver and lymph nodes of the HCMV-infected mice. Treatment with clinical grade G-CSF (2,5mg/mouse/day) for seven days furthermore increased signal. Viral genomic copies were detected in spleen, liver and bone marrow of infected mice. Furthermore infection was detected in CD34+ cells from bone marrow and in CD169+ macrophages from spleen. Infection with HCMV leads to and expansion and maturation of memory T cells and reactivation leads to an increased PD-1 expression on T cells. Further HCMV infection lead to B cell maturation by increased numbers of memory B cells in spleen and bone marrow, with production of HCMV specific antibodies.
Conclusion: Long-term reconstituted humanized mice infected with HCMV displayed a robust, yet complex and dynamic spatial modulation of several human adaptive immune responses. Compared with previous approaches, this model is relatively straightforward and can be used broadly to test the efficacy and mode of action of novel therapies.

[1] Sinzger *et al.*, 2016, Generation of a Gaussia luciferase-expressing endotheliotropiccytomegalovirus for screening approaches and mutant analyses, Journal of Virological Methods, 182–189

ANTIVIRAL CHEMOKINE INTERFERON GAMMA-INDUCED PROTEIN 10 (IP-10) RESPONSE INDUCED BY RESPIRATORY SYNCYTIAL VIRUS (RSV) AND THE TLR3 AGONIST POLY I:C IN PRECISION-CUT LUNG SLICES

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Respiratory Syncytial virus (RSV) is a common agent affecting lower airways in children with increasing evidence for higher risk of subsequent asthma. RSV infection is characterized by the cytopathic effect of syncytial formation and triggers the innate immune response to release cytokines and chemokines, e.g. interferon gamma-induced protein 10 (IP-10/CXCL10). This virus- induced inflammation can also be mimicked by the toll-like receptor 3 agonist Poly I:C. In this work, we hypothesized that RSV could infect precision-cut lung slices (PCLS) from humans and non-human primates, producing an antiviral response comparable to poly I:C.

PCLS containing airways were prepared from lung sections of human and non-human primates (Rhesus, *Macaca mulatta,* and Cynomolgus, *Macaca fascicularis*). PCLS were inoculated with human-RSV-A2, UV-inactivated RSV, or medium. Macaque PCLS were incubated up to 5 days post-infection (dpi) and human slices for 1 dpi. Macaque PCLS were also incubated with poly I:C at 100 µg/ml. Viral load, tissue viability, and immune response assays were assessed in supernatants, lysates, or slices.

The inocula infectivity of 10^6 IU/ml and UV-inactivation was confirmed by $TCID_{50}$ assay on Hep-2 cells. Virus replication was determined by immunofluorescence staining using an anti-RSV FITC- labeled monoclonal antibody in PCLS, demonstrating the presence of RSV-infected cells spread over the tissue. RSV slightly reduced tissue vitality, determined by live/DEAD® and LDH assays. Virus-induced mediator release in PCLS was assessed by ELISA. Infection of PCLS with RSV at 10^6 IU/ml significantly increased the release of the antiviral chemokine IP-10 in NHP-PCLS, reaching a 3.5-fold increase at day 1 and 2.7-fold increase at day 4. At day 5, the increase was not significant. RSV and poly I:C produced similar IP-10 response, when compared to vehicle controls: both stimuli induced a 4.0-fold increased IP-10 production in rhesus PCLS and 3.0-fold in cynomolgus PCLS. In human-PCLS, RSV infection provoked a 39.8-fold increased IP-10 release, representing about 11 times more response than in macaque-PCLS.

RSV infects lung tissue ex vivo. Although non-human primates are not a natural host for RSV, lung slices provided from rhesus macaque could be infected, presenting antiviral immune response comparable to human PCLS. The IP-10 response caused by RSV is comparable to the viral surrogate poly I:C. The investigation of ex vivo RSV infection in lung tissue of different species is the first step towards a model for preclinical pharmacological investigations which resembles the human situation.

43 GENETIC DETERMINANTS OF SEVERE RESPIRATORY SYNCYTIAL VIRUS INFECTIONS IN INFANTS

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Respiratory syncytial virus (RSV) is the most common cause of lower respiratory tract infections in infants. Disease severity varies widely among children and ranges from mild upper respiratory symptoms to severe bronchiolitis. Genetic factors governing disease severity are incompletely defined.

101 children aged between 0-2 years and suffering from severe acute RSV infections were subjected to whole exome sequencing (WES). Since interferon-regulated immune responses are critical for the defense of RSV infections and the course of the disease, we focused on 5142 genes that either trigger expression of interferons, or contribute to interferon signaling or are controlled by interferons. In total 30,039 variants mapped to these genes. Heterozygosity and homozygosity counts in our cohort as well as in an ethnically matched sub-cohort of the Exome Aggregation Consortium (ExAC) were used to calculate the significance of association of variants with severe RSV infection. Collectively, 218 coding polymorphisms mapping to 84 genes were significantly associated with severe RSV infection. Associated genes expressed in primary human airway epithelial cells were silenced and the impact on RSV infection was quantified. Moreover, their expression upon RSV infection of air-liquid interface cultures of human airway epithelial cells was quantified with single cell resolution. More than six novel viral restriction or dependency factors were identified including proteins involved in cellular ER-stress response and regulation of ER-associated protein degradation (ERAD), in inflammatory cytokine signaling and a protein kinase activated by double-stranded RNA which mediates the effects of interferon in response to viral infection.

This integrated approach combining clinical phenotyping, WES, variant calling/association and functional screening provides a new paradigm for discovery of genetic traits and protein functions affecting the course and outcome of infectious diseases.

RESISTANCE OF SH-SY5Y NEUROBLASTOMA CELLS TO FILOVIRUS CELL ENTRY SUGGEST THE EXISTENCE OF UNIDENTIFIED HOST FACTORS CRITICAL FOR INFECTION AND NEW DRUGGABLE TARGETS FOR ANTI-FILOVIRAL THERAPY

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Ebola virus disease (EVD) was the cause of the infamous 2013-2016 West African outbreak. Although in early stages of infection macrophages, monocytes, and dendritic cells are the primary target, filoviruses have a wide cell tropism. They are able to infect virtually any cell type with the exemption of cell lines of lymphocytic origin (Wool-Levis and Bates. 1998) and later confirmed *in vivo* (Geisbert *et al.* 2000). Using a filovirus susceptibility assay in a panel of cell lines we identified SH-SY5Y, a neuroblastic non-lymphocytic cell line, as an uncharacterized cell line refractory to filovirus entry. These results were later validated by rVSV-EBOV-GP and authentic filovirus infection. Characterization of the cell line revealed that 1) resistance was not related to expression levels of previously reported factors, 2) intracellular factors cathepsin B and L, NPC1, and TPC1-2 were functional in SH-SY5Y cells, 3) filovirus cell entry was not inhibited by a dominant restriction factor, and 4) a GP1- hFc fusion protein containing the Receptor Binding Domain (RBD) failed to bind to SH- SY5Y´s cell. Delivery of a cDNA library from highly susceptible cells renders SH-SY5Y cells susceptible to filoviral cell entry. Taking together, these data suggest the existence of proteinaceous unknown panfilovirus specific host factors that play an essential role in viral entry and hence, new vulnerable steps in the virus replication cycle for drug development.

45 RESCUE EBOLA PATIENTS *IN SILICO*

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We developed a mathematical model of immune responses to Ebola infections, based on the available cytokine response data from asymptomatic, survival and fatal patients in the two Ebola outbreaks in Gabon 1996. We found that TNF-α can explain subsequent cytokine responses and allows to quantitatively stratify patients into survivors and fatal cases. The model suggested that the virus escaped from being detected in the early phase in the fatalities while in the survivors the virus was detected but the virus suppressed the magnitude of the immune response. The model further suggests that antibodies provide the key resolving mechanism in survivors while IFN-γ is the key defense mechanism in fatal cases. The above findings allow us to propose a cytokine interference strategy to rescue fatal Ebola infections *in silico*.

MOLECULAR BASIS OF NON-RESPONSIVENESS TO HEPATITIS B VACCINATION

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After a complete cycle of three vaccinations against the hepatitis B virus (HBV), the majority of people show an anti-HBs antibody titer of more than 100 IU/L for many years, whereas approximately 5% of the people do not mount virus-specific antibody responses and are so-called non-responders. A titer of 100 IU/L and higher is known to be protective against the highly infectious virus. In contrast, the titer of non-responders stays permanently lower than 10 IU/L, which constitutes a serious challenge for health-care workers, who have a high risk to get in contact with contaminated blood. In the case of influenza vaccination, the phenomenon of non-responsiveness increases with advanced age. In contrast, the group of HBV non-responders includes young and otherwise immunocompetent and healthy people. Although the phenomenon is known for many years, the molecular basis of HBV vaccination non-responsiveness is not fully resolved, yet.

We initiated an observational study to monitor the immune reactions after HBV vaccination to reveal differences in the immune responses of non-responders and responders. This immunomonitoring includes a cytometer-based analysis of blood samples to analyze the distribution of immune cell subsets as well as their activation status. Additionally, cytokine responses of responders and non-responders are studied from blood. The second arm of the project includes whole genome sequencing, which will allow the search for genetic markers that correlate with non-responsiveness to HBV vaccination. The objective is to identify biomarkers that will allow prediction of HBV vaccination non-responsiveness. On the basis of this information, we plan to develop improved vaccination strategies.

47 HUMAN CYTOMEGALOVIRUS PUL11 INDUCES ANTI-INFLAMMATORY IL-10 SECRETION THAT IS DEPENDENT ON HOST CD45 SEQUENCE

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Human cytomegalovirus (HCMV) is a ubiquitous human pathogen, infection with which can have serious consequences for individuals with suppressed or immature immune systems. Congenital infections or infections in transplant recipients can lead to disability, transplant rejection, an increased rate of secondary infections and death.

HCMV is a master manipulator of the host's immune system, allowing it to evade and suppress key immune responses and thereby to establish persistent infections. Infected immune cells are modulated to benefit the virus and non-infected lymphocytes can also be affected, by direct contact with infected cells or via secreted products.

One such mechanism is the manipulation of T cell cytokine production. We have shown that the variable HCMV protein pUL11 induces the secretion of the anti-inflammatory cytokine IL-10 from T cells via a direct interaction with the CD45 phosphatase on the surface of uninfected T cells. IL-10 secretion is upregulated during active infection and has been suggested to be an important driver of HCMV immunomodulation, facilitating virus replication by inducing an anti-inflammatory environment. IL-10 levels have been shown to correlate with HCMV viral load in transplant recipients.

Interestingly, variations in both host and viral factors may contribute to the function of pUL11. We have shown that a polymorphism in host CD45 (C77G) abrogates the response of T cells to pUL11, meaning that the induction of IL-10 secretion is greatly reduced in these CD45 variant cells. This suggests that this CD45 polymorphism should be evaluated as a diagnostic marker to predict variations in clinical outcome in HCMV-reactivating immunocompromised patients.

The UL11 gene is highly variable and can be classified into seven different genotypes. In stem cell transplant recipients, genotype 1 of UL11 appears to be associated with later reactivation of HCMV, which may indicate functional variation between the genotypes.

A deeper knowledge of the mechanisms by which HCMV induces immunosuppression in the host will allow us to understand the contribution of individual host factors and strain specific viral variations to the outcomes of this clinically important infection. These insights will then help to improve diagnostic techniques and treatment decisions for these vulnerable patients.

NOTES

INFORMATION

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CiiM CENTRE FOR INDIVIDUALISED INFECTION MEDICINE

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